European Cooperation in Science and Technology
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Secretariat

Brussels, 2 July 2010

COST 4131/10

MEMORANDUM OF UNDERSTANDING

Subject :	Memorandum of Understanding for the implementation of a European Concerted Research Action designated as COST Action BM1002: Nanomechanics of intermediate filament networks - NANONET

Delegations will find attached the Memorandum of Understanding for COST Action BM1002 as approved by the COST Committee of Senior Officials (CSO) at its 178th meeting on 25 May 2010

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MEMORANDUM OF UNDERSTANDING

For the implementation of a European Concerted Research Action designated as

COST Action BM1002

NANOMECHANICS OF INTERMEDIATE FILAMENT NETWORKS - NANONET

The Parties to this Memorandum of Understanding, declaring their common intention to participate in the concerted Action referred to above and described in the technical Annex to the Memorandum, have reached the following understanding:

1. The Action will be carried out in accordance with the provisions of document COST 4159/10 "Rules and Procedures for Implementing COST Actions", or in any new document amending or replacing it, the contents of which the Parties are fully aware of.

2. The aim of the Action is to establish a scientific platform to take full advantage of the emerging knowledge on intermediate filaments and novel developments in cell biology, molecular biology, (bio)chemistry, engineering, mathematics and physics in appropriate commercial and medical applications.

3. The economic dimension of the activities carried out under the Action has been estimated, on the basis of information available during the planning of the Action, at EUR 44 million in 2010 prices.

4. The Memorandum of Understanding will take effect on being accepted by at least five Parties.

5. The Memorandum of Understanding will remain in force for a period of 4 years, calculated from the date of the first meeting of the Management Committee, unless the duration of the Action is modified according to the provisions of Chapter V of the document referred to in Point 1 above.

A. ABSTRACT AND KEYWORDS

NANONET will establish the first scientific platform to focus and integrate the exciting new knowledge on intermediate filaments with novel developments in cell biology, molecular biology, medicine, (bio)chemistry, engineering, mathematics and physics. Intermediate filaments are a key component of the cytoskeleton integrating the full spectrum of cellular responses to biochemical, biomechanical and cellular stresses. Given the substantial European expertise in the biology and physics of intermediate filaments, this COST Action to form NANONET will enhance the integrative research discussion between groups and provide an official forum for the first time for this strategically important sector of European science.
It will enable the synergistic development of novel collaborative research projects to deliver a multi-disciplinary understanding of cell homeostasis and its vulnerabilities in health and disease. NANONET will elevate and promote awareness of these important nanofilaments, increasing knowledge of their nanomechanical and biological properties from which new therapies and treatments for the health and well-being of the European people will emerge. A COST Action will capture the commercial applications of this new knowledge and take advantage of current scientific advances in the field and therefore draw in experts, students and industrialists from biology, chemistry, biomedicine, engineering, mathematics and condensed matter physics.
Keywords: intermediate filament cytoskeleton and associated proteins, bio- and cell mechanics, mechanotransduction and signalling platforms, protein polymer physics, materials science and renewable sources.

B. BACKGROUND

B.1 General background

From microbes to man for cells to have shape, form and function they need a dynamic, internal skeleton. This skeleton is known as the cytoskeleton which in vertebrates comprises three major filament systems: microfilaments, microtubules and intermediate filaments. Whereas microfilaments and microtubules serve as tracks for motor proteins and are engaged in cell motility, intermediate filaments are fundamentally engaged in determining cellular plasticity. Intermediate

filament proteins form intracellular polymeric filaments with a diameter of around 10 nm that are essential for the cell's function and structure. The intermediate filament cytoskeleton transduces mechanical and biological stimuli into molecular responses. It conveys resistance against mechanical stress, thereby preventing structural disintegration of the cell. Moreover, it integrates signalling and transport processes in cells. Mutations in intermediate filament genes are the cause of a broad range of diseases, including neurological, heart and skin disorders, and can also determine the organism's response to diseases. The filamentous cytoskeleton of intermediate filaments connects the cell membrane to cellular organelles and to the nucleus. This intermediate filament cytoskeleton, which is dynamic and tailored to deal with mechanical stress, has recently emerged as (i) a signalling platform in stress and mitogenic signalling pathways, as well as (ii) an organizer of a number of associated proteins (chaperones, molecular motors and kinases). A recent overview of these functions can be found in a dedicated series of reviews in the *Journal of Clinical Investigation*, 119 (7) 2009.

Novel developments in biological, physical and chemical technology have contributed to our recent increase in knowledge on the nanomechanics and biological function of the intermediate filament cytoskeleton. Despite this recent progress, there is still a lack in understanding how the intermediate filament cytoskeleton contributes to mechanical resistance and mechanoresponsiveness and how mutations in the intermediate filament genes can lead to disease. A particular challenge in the intermediate filament field is to develop targeted therapies for the different intermediate filament based diseases, which is currently lacking. Development of such therapies is greatly dependent on a full comprehension of the function and nanomechanics of the different highly specialized intermediate filament cytoskeletons. From a materials perspective, intermediate filaments have remarkable mechanical properties, being exceptionally strong, tough, and resilient. If the structure/function relation is resolved, this knowledge can be translated to the rational design of protein- or peptide-based materials for demanding applications such as drug delivery and tissue engineering.

A COST Action would greatly facilitate the integration of the newly acquired knowledge to capture the commercial applications of this new knowledge, take advantage of current scientific advances in the field and draw in experts and students from engineering, mathematics and condensed matter physics. A concerted exchange of such knowledge, with the intermediate filament cytoskeleton as the central theme, is not only timely, but also essential since it can provide an interdisciplinary

research platform to inspire new research initiatives and commercial applications. To pursue this goal a COST Action offers the appropriate framework. Specific Working Group meetings, NANONET meetings and Short-Term Scientific Missions will allow us to integrate already ongoing research projects by facilitating networking activities, and also to acquire multi-disciplinary skills and competence needed to incite the development of therapies for intermediate filament based diseases and promote commercial exploitation of this new knowledge. By coupling the strengths of the individual research projects, funded by national and international funding agencies, the Action will optimize an exchange of knowledge, expertise and resources, and prepare for new research initiatives. Moreover, NANONET will be instrumental in capitalizing on the existing strengths of the European intermediate filament research in the broadest sense, from biological function to nanomechanics, and in attaining a world-leading position of the European research groups in this research field.

B.2 Current state of knowledge

The intermediate filament cytoskeleton enables cell-type specific functions. This is because in man the intermediate filament gene family comprises 70 members that are differentially expressed during development and in distinct tissues. In addition, it has recently been shown that splice variants of intermediate filament proteins are expressed, adding a level of complexity to this system, which is likely to confer further distinct cell or tissue specific functions. This is in sharp contrast with the components of the microtubule and the actin cytoskeleton which are more or less ubiquitously expressed in all cell types. The 70 different intermediate filament proteins form highly specialized polymeric filamentous networks, such as keratins in skin, vimentin in mesenchymal and lens cells, neurofilaments, GFAP, alpha-internexin and synemin in the central nervous system, desmin and syncoilin in muscle, peripherin in the peripheral nervous system and nestin in neural stem cells. The long polymeric filaments are linked to integrin complexes within the cell membrane through plakins and are coupled to the nuclear membrane by nesprins. This unique feature of intermediate filaments, to connect the cell membrane to the cell nucleus, makes these structures well positioned to transduce biomechanical signals into signalling events. The intermediate filament cytoskeleton is now regarded as the main candidate for regulating a proper cellular response induced by a physiological mechanical stimulus (e.g. muscle contraction) or a pathological process (e.g. brain injury).

Mutations in intermediate filament genes are the cause of many different devastating disorders. Even though these are mainly defined as rare diseases, since they affect less than 1 in 2000 citizens in Europe, they pose an enormous burden on the patients and their caretakers as these diseases are often chronic, progressive, degenerative and life-threatening. Examples are Alexander's disease, a fatal neurodegenerative disease caused by mutations in GFAP, and cardiac and familial cardiac and skeletal myopathy caused by mutations in desmin and plectin. Unfortunately, effective therapies for these diseases are currently non-existent and highly sought after. In addition, problems with the intermediate filament associated protein filaggrin are the cause of the extremely common and chronic diseases asthma and eczema. These diseases are manifestations of the loss of skin barrier function. Fundamental insights into the function and nanomechanics of the intermediate filament cytoskeleton will provide an innovative knowledge base for the development of treatment of the different intermediate filament based diseases.

Despite the broad variety of intermediate filament proteins expressed in different tissues, there is a high level of similarity in the structural design of the intermediate filament cytoskeleton. The monomers making up these protein filaments do differ in their amino acid sequence, but share similar protein domain motifs, as they all consist of a central alpha-helical rod flanked by flexible and highly variable N- and C-termini. All intermediate filament proteins form networks following a similar, hierarchical assembly scheme. The self-assembled filaments also share similar mechanical properties: the filaments are exceptionally strong and extensible, and form networks that are highly elastic, tough, and resilient. For this reason, intermediate filaments are believed to play a key role in protecting cells from excessive deformation. Understanding the structure/mechanics relation for different intermediate filament proteins, will allow the extraction of bio-inspired design principles to build new materials that are stronger than conventional man-made plastics, yet built from renewable, bio-friendly resources (proteins or (synthetic) peptides). Due to its multi-disciplinary constitution, NANONET will be in a unique position to translate fundamental insights into intermediate filament proteins and their biological function into the rational design of new commercial materials and biomimetics.

Currently, several laboratories world-wide are working on a broad array of research questions all focused on the intermediate filament cytoskeleton. In Europe a multi-disciplinary research cluster is emerging, consisting of excellent established and outstanding Early-Stage Researchers which are all involved in intermediate filament related research (see also D). These researchers have started to

build informal ties. Now it is timely to build a formal network which brings these researchers together and enables them to have sufficient critical mass to perform world-leading research, to translate this into clinical and engineering applications, and to achieve effective outreach activities. The innovation of the COST Action is to bring together, on a regular basis, European researchers and their industrial contacts with multi-disciplinary backgrounds, all with a shared interest in the intermediate filament cytoskeleton in the broadest sense. The COST Action will promote scientific and technical discussions and stimulate the exchange of expertise and technology, will advance the knowledge on the intermediate filament cytoskeleton and will perform outreach activities to increase awareness of the importance of this key component of the cytoskeleton. In view of the current developments in the field, it is surprising that such a networking Action with the intermediate filament cytoskeleton as a central theme is still non-existent.

B.3 Reasons for the Action

Numerous groups in Europe are performing cutting-edge research of the highest impact on multiple aspects of the intermediate filament cytoskeleton. Since intermediate filaments play interrelated roles in biochemical signalling, mechanical protection, and mechanoresponsiveness, intermediate filament research is by nature multi-disciplinary. It involves expertise and techniques from physics, mathematics, biochemistry, molecular biology and cell biology. Moreover, since intermediate filaments are central to the function of many tissues, intermediate filament research also relates to various clinical disciplines, including neurology, neurorehabilitation, cardiology, ophthalmology and dermatology. Up to now European research groups have played a leading role in initiating such cross-disciplinary initiatives. Moreover, this field of research gained interest of several highly talented “Early-Stage Researchers” from different disciplines who, together with the established researchers, are enthusiastic and dedicated to intensify the European scientific interaction on this topic.

NANONET strives to unite these efforts and to facilitate technical and scientific interactions between different researchers from across the science disciplines from mathematics to medicine to encourage interdisciplinary approaches to understand the underlying biological principles of cell homeostasis in health and disease. NANONET will ensure that this new knowledge will be effectively exploited commercially and utilised to increase the health and wellbeing of people in Europe. This novel COST network will also actively interact with clusters of excellence in the US,

Australia, New Zealand, Canada, Japan and Singapore. The main aim is to increase the knowledge on the biological function and the nanomechanics of the cytoskeletal intermediate filament network, and to engage a broader community of biologists, clinicians, physicists, and materials scientists. The current group of researchers, that are committed to participate in this new COST Action, are excellent researchers and trained physicists, chemists, biochemists, molecular and cell biologists, and medical doctors from 11 COST countries.

The immediate benefits of NANONET will be the strengthening of existing and promotion of novel cross-disciplinary initiatives by stimulation of the scientific communication between research groups and facilitation of exchange of knowledge, expertise and resources. The future benefits of NANONET will be a strong European network of intermediate filament scientists which is able to compete successfully for dedicated European funding (FP7), which has an active interaction with clusters of excellence world-wide, and which offers excellent multi-disciplinary research and training opportunities with state-of-the-art technology for talented young scientists. Finally, and most importantly, our combined efforts will lead to a gain in knowledge on the intermediate filament cytoskeleton, which is a requisite for the discovery of new leads to develop novel therapies for patients suffering from intermediate filament based diseases or carrying intermediate filament mutations that modify their response to disease and for the development of novel biomimetic materials, which are biodegradable, renewable and intermediate filament network inspired.

This COST Action, NANONET, is mainly aimed at scientific/technological advance. However, an increased understanding of the function of mutated intermediate filament proteins in intermediate filament based diseases is expected to lead to the development of novel therapeutic strategies for these diseases. In this sense, NANONET is also aimed at reducing the societal and economic burden of caring for people with an intermediate filament based disease and to further address the role of intermediate filaments in the disease response. Furthermore, knowledge on the physical properties and nanomechanics of intermediate filaments will contribute to green technology, as it will inspire the development of environment friendly biodegradable materials. This technological advancement will reduce the societal and economic burden of pollution by nondegradable plastics, which are based on natural oil.

B.4 Complementarity with other research programmes

NANONET aims to integrate activities in the full spectrum of science disciplines – biology, chemistry, biomedicine, computer science, engineering, mathematics and physics – integrating a multidisciplinary approach to understand the biological basis of the many human diseases caused by mutations in intermediate filament proteins themselves or their associated proteins. There is currently no comprehensive European research program focused on the biology, physiology, and biomechanics of the intermediate filament cytoskeleton in health and disease.

C. OBJECTIVES AND BENEFITS

C.1 Main/primary objectives

The main objective of the Action is to establish a scientific platform to take full advantage of the emerging knowledge on intermediate filaments and novel developments in cell biology, molecular biology, (bio)chemistry, engineering, mathematics and physics in appropriate commercial and medical applications. The expected deliverables are: [1] Workshops: Informal meetings which will be jointly organized yearly by the different Working Groups and which are open for all principal investigators, senior scientists, post-docs and students from the participating labs and interested commercial partners. [2] Short-Term Scientific Missions: Exchange visits available for PhD students and post-docs to acquire novel techniques and multi-disciplinary experience. [3] Training school: Organized every 2 years for European PhD students and post-docs. This school will provide multi-disciplinary training at the interface of cell biology, biophysics and clinical aspects of the intermediate filament cytoskeleton. The school will also be open for PhD students and post-docs from outside Europe, but they will not be financed from the COST Action. Non-COST world-leading researchers will be invited to teach. [4] Strategic meetings: Organized yearly (combined with a Workshop, Training school or Management Committee meeting) to prepare for writing EU proposals and programs. [5] Final conference: Non-COST world-leading keynote speakers will be invited to create international exposure and to connect with non-European clusters of excellence.

C.2 Secondary objectives

The secondary objectives are categorized into scientific, networking and other objectives.
The scientific objectives of NANONET are:

- to integrate and increase knowledge on the function of the intermediate filament cytoskeleton in the biology of the cell
- to integrate and increase knowledge on the nanomechanical properties of the intermediate filament proteins and cytoskeleton
- to develop novel biomimetic materials based on renewable sources, inspired by the properties of intermediate filaments and their networks
- to understand how intermediate filament proteins contribute to health and disease, which will lead to the discovery and development of new leads and therapies in drug delivery, stem cell and regenerative medicine, and of course the treatment of intermediate filament based diseases
- to understand how intermediate filaments determine the response to cellular stress and relevant diseases

The networking objectives of NANONET are:

- to stimulate, to formalize, and broaden the existing and developing collaborations between the different research groups
- to promote interdisciplinary interactions
- to train young scientists for this multi-disciplinary field of research
- to increase awareness of the important function of the intermediate filament cytoskeleton among biologists, clinicians, physicists and materials scientists
- to prepare collaborative research Actions
- to stimulate joint interactions with intermediate filament researchers outside Europe

The other objectives are:

- to establish a broad awareness of the important role of the cell's intermediate filament cytoskeleton in health and disease (outreach)
- to actively pursue a gender balanced science network

- to help young researchers to build up an international network and to achieve international recognition
- to develop interdisciplinary approaches and multidisciplinary teams to maximise the generation of new knowledge and its commercial and medical exploitation
- to develop links with chemical and processing industry to drive the commercial exploitation of the new knowledge emerging from our project

In quantitative terms the secondary objectives can be evaluated by assessing the number of:

- active participants in NANONET
- scientific meetings organized by NANONET
- Short-Term Scientific Missions supported by NANONET
- workshops organized by NANONET
- non-COST scientists involved in meetings organized by NANONET
- young researchers attending the NANONET workshops
- young researchers in Management Committee of NANONET
- female scientists in Management Committee of NANONET
- female scientist attending NANONET workshops and applying for Short-Term Scientific Missions
- joint publications of scientists involved in NANONET
- collaborative research proposals of scientists involved in NANONET
- visitors of the NANONET intermediate filament website

C.3 How will the objectives be achieved?

The means needed to achieve the objectives of NANONET are:

- Formation of a competitive network of leading international scientists with a common interest in the intermediate filament cytoskeleton
- Establishing an infrastructure for regular scientific meetings
- Sharing expertise, reagents and resources, Teaching and training young researchers by exchange visits and through training schools

- Inviting intermediate filament researchers from non-COST countries to the European work meetings, training courses and final conference.
- Setting up a NANONET website

The instruments used to achieve these goals are:

- Network meetings, scientific meetings and workshops
- Working Groups
- Short-Term Scientific Missions
- Training courses

The deliverables are (see time table in F):

- Establishing a Management Committee and Working Groups
- Developing a concept for the integration of European research activities into international activities
- Organisation of network meetings, Management Committee and Working Group meetings, scientific workshops and meetings
- Organisation of training schools
- Establishing a platform for exchange visits for the career development of young researchers and women
- Establishing a NANONET website

C.4 Benefits of the Action

Research initiatives on the intermediate filament cytoskeleton are both timely and appropriate given this sector has received no European Action to date to disseminate, or to promote or to commercialise this important science sector. Besides the facilitation of networking activities, the Action also envisions additional benefits such as:

- Integrated novel knowledge on the function of the intermediate filament cytoskeleton in biomechanics and mechanotransduction
- Technological development of future novel biomimetics

- Establishment of awareness for the important role of the cell's intermediate filament cytoskeleton in health and disease
- Discovery of new leads to develop novel therapies for patients suffering from intermediate filament based diseases
- Training platform for young scientists on an integrated, European level

C.5 Target groups/end users

NANONET activity will target all major scientific groups in Europe that work in the field of intermediate filaments and associated systems. These groups will be the end users for which NANONET aims to facilitate and establish a scientific platform to achieve the convergence of the emerging knowledge on intermediate filaments with novel developments in cell biology, molecular biology, (bio)chemistry, biomedicine, engineering, mathematics and physics and their commercial and medical application. More specifically, the objectives are targeted to the following users: (i) young researchers with an interest in intermediate filament biology and biophysics, (ii) patients suffering from intermediate filament based diseases, (iii) the European research community interested in intermediate filament biology and biophysics, (iv) chemical, pharmaceutical, processing and biotechnology industry interested in further developing drugs to treat intermediate filament based diseases, and (v) scientists and industry interested in bio-inspired functional materials from renewable resources. The international outreach activities will target the scientific community world-wide and contribute to the international discussion and interaction. Together this will be seminal in determining future research infrastructures and programmes.

D. SCIENTIFIC PROGRAMME

D.1 Scientific focus

NANONET research projects will be centered around the physiology, biology, biomedicine and physics of the intermediate filament cytoskeleton in different tissues. Bringing together for the first time scientists and their knowledge from different fields and disciplines is the main innovative aspect of this Action.

NANONET will establish the following Working Groups (WGs) to achieve the scientific, networking and outreach activities described in C2.

- WG 1 - Mechanotransduction and cell signalling
- WG 2 - Nanomechanical properties and impact on cell mechanics
- WG 3 - Translation to bio-inspired materials
- WG 4 - Intermediate filaments in health and disease
- WG 5 - Outreach

The main research questions are:

1. What are the molecular mechanisms of intermediate filament cytoskeleton assembly in simplified, reconstituted model systems and in cultured cells?
2. What is the detailed 3D structure of the intermediate filament cytoskeleton at the nanoscale?
3. What is the hierarchical intermediate filament structure at different length scales, from molecular (protein building block) to fiber and finally to mesoscopic (network) scale?
4. What are the mechanical properties of intermediate filaments in a reconstituted system, and what is the contribution of this cytoskeleton to the mechanical properties of a cell or tissue?
5. How can the intermediate filament polymeric network formation be translated into the development of novel synthetic macromolecules with a (biological) application or function?
6. What is the role of intermediate filaments in cellular mechano-responsiveness, where intermediate filaments' extracellular events are linked to molecular responses in the cell nucleus?
7. How do mutations in intermediate filament proteins lead to cellular dysfunction in the central nervous system and heart tissue?
8. What is the role of the cytoskeleton (especially the intermediate filament proteins keratin, synemin, nestin, vimentin and GFAP) in the regulation of mechanics and motility of cancer cells, neural stem cells and astrocytes?
9. How can the intermediate filament system be modulated in experimental disease models in order to develop therapeutic strategies to combat intermediate filament-based diseases and to improve the body's own defence mechanisms?

NANONET will be open to include other intermediate filament related research and experimental approaches if asked for from the European research community in the future. Additional Working Groups can also be added if required.

D.2 Scientific work plan methods and means

NANONET will create a network of basic, pre-clinical and clinical scientists, with expertise in intermediate filament cytoskeleton formation and function. Its objectives will be achieved by organizing regular scientific meetings, stimulating short exchange visits between labs, and promoting our research and Actions through a website dedicated to the intermediate filament cytoskeleton. Thus, NANONET will contribute to the emerging biomechanical, cellular, and molecular knowledge on the intermediate filament cytoskeleton and accomplish awareness for its important role in health and disease.
The rationale and specific objectives for each Working Group and the interactions with the other Working Groups are as follows:

Working Group 1: Mechanotransduction and cell signalling
Rationale: Cells in tissue "sense" their environment and respond to externally applied forces. The intermediate filament cytoskeleton connects the extracellular matrix with the cell's nucleus and therefore is tailored to transduce mechanical force and biochemical signals into a biological response. Complex intracellular pathways transmit this information towards the nucleus leading to the launch of transcriptional programs. The composition of the cytoskeleton is likely to be instrumental in the propagation of the external force to the nucleus. Especially, intermediate filaments have been shown to form direct physical linkages via versatile cytolinker proteins such as plectin, from the cell's nucleus to integrin adhesions, which in turn further connect to the extracellular matrix. The mechanism of force sensing seems to be particularly important for controlling embryonic development and stem cell differentiation.
Defects in the nuclear envelope, a structural barrier that enwraps the genetic material, cause a multitude of genetic diseases that include skeletal muscle, adipose tissue, and skin tissue degeneration, heart muscle failure, and even premature ageing. While the nuclear envelope is crucial it remains unclear why this is the case. The nuclei are likely the "control centers" of the eukaryotic cell, not only because they contain the genetic material but also due to their capacity to

signal and to influence several intra- and extracellular processes. Central elements of this signalling platform are the nesprins. Nesprins reside at the outer nuclear membrane and connect the nucleus with the cytoskeleton in organisms as diverse as yeast, amoebas, plants, worms, flies and vertebrates. In addition, nesprins form a complex with the inner nuclear membrane SUN proteins, and the nuclear lamins, thus providing a link between the Nucleoskeleton and Cytoskeleton (LINC complex).

The experimental methodologies brought together in this WG by NANONET include gene expression profiling (DNA microarrays, quantitative real time polymerase chain reaction, including single cell rtPCR) and proteomics), the documentation of cellular architecture defects and the monitoring of transcompartimental signalling events using state of the art imaging techniques (total internal reflection fluorescence and spinning disc microscopy) on cells in which the intermediate filament cytoskeleton is genetically modified (mutations) or in which components of this network are silenced (short interfering RNAs and knockout). Also immortalized and primary cell cultures of genetically modified mouse lines are available.

This Action will bring together expertise on different aspects of mechanotransduction and cell signalling pathway from physics to molecular and cellular biology, which allows an optimal exchange of resources and knowledge.

Objective: To elucidate the process of force transduction within the cell from the external mechanical stimulus to the molecular response in the nucleus.

Interactions with other Working Groups: The understanding of the scaffolding and signalling principles in WG1 will reveal the key principles directing transient associations and the formation of nanomachines in the cell to develop industry links in WG5. Knowledge from WG2 is needed to provide a basic understanding of the mechanics of the intermediate filament network. And knowledge generated in WG1 will help to understand the function of intermediate filaments in health and disease in WG4.

Working Group 2: Nanomechanical properties and impact on cell mechanics

Rationale: Intermediate filaments and networks thereof are extremely resistant to breakage and can be stretched up to three-times their length in a resting state. The key to this extreme stretchability of the filaments is the hierarchical structure, which facilitates cascaded deformation mechanisms at different levels of strain. As physical attributes of whole cells are always influenced by numerous

components and parameters, investigation of purified *in vitro* networks is a valuable prerequisite for a better understanding of the material properties of biopolymer networks in living cells. The Action will therefore combine studies on reconstituted intermediate filament proteins and networks with studies involving whole cells. In reconstituted systems NANONET has access to all relevant length-scales, while in whole cells interactions of intermediate filaments with other cytoskeletal systems, the membrane, nucleus, and organelles are investigated. Data from these studies will be a basis for mechanical modelling of intermediate filament networks.

The relevant length scales for cells and cellular components are nanometers and micrometers. Therefore, to probe such systems tools on exactly these length scales are required. A variety of such techniques exists in the NANONET laboratories. The experimental techniques in NANONET range from probing molecular length scales (X-ray scattering and diffraction) to the fiber scale (microscopy, atomic force (AFM) and electron microscopy) and the network scale ((micro-) rheology, optical tweezers microfluidics). AFM methods can test individual filaments but also whole (adherent) cells. By contrast, for suspended cells an optical stretcher, a dual-beam laser trap for contact-free deformation of individual cells is available. Microfluidic methods are used to probe both adherent and suspended cells. These experiments will provide the Action with a multi-scale understanding of structure-mechanics relation in intermediate filament networks and whole cells. One vision of this bottom-up approach is to understand the mechanics of cellular components well enough to be able to build “artificial cells” by incorporating proteins in liposomes. (Statistical) mechanics is one important aspect of cellular physics, the other being active processes such as transport by motors and polymerization and depolymerization regulated by (de)phosphorylation. Furthermore, the mechanical models, computer simulations and analytical calculations of intermediate filament networks will provide a deeper understanding of celluar mechanics acros different length scales. The biological expertise within NANONET will provide the biologically defined, recombinantly bioengineered proteins to study the nanofilaments in solution and in cells allowing a multiscale modelling approach from theoreticians with appropriate physics and mathematical skills. This will contribute to the understanding and definition of the physical properties of the nanofilaments that comprise the cytoskeleton of mammalian cells. Ultimately, alterations in cell mechanical properties caused by intermediate filament based diseases could also be used as a novel diagnostic or prognostic marker.

Objective: To understand the mechanical properties of intermediate filaments in a reconstituted system, and the contribution of this cytoskeleton to the mechanical properties of a cell or tissue.

Interactions with other Working Groups: Knowledge generated in WG2 will help to understand the function of intermediate filaments in health and disease in WG4 and will inspire the development of novel biomimetic materials in WG3. The understanding of the nanomechanical properties will reveal fundamental properties of intermediate filaments needed to develop industry links in WG5.

Working Group 3: Translation to bio-inspired materials

Rationale: Intermediate filaments are the ultimate nanofilaments and their innate properties will inspire important new technologies and may potentially fashion the development of a new generation of smart materials. NANONET will achieve this goal by close cooperation with industry, including small to medium enterprises (SMEs – see part IIB), using our new knowledge and technology to draw in new industrial partners and so increase the potential commercial application of our project. The component proteins of intermediate filaments make some of the most resilient filaments encountered in biology and are, for instance, the staple component of hair, hoof and skin. The spectrum of biomechanical properties in each of these quite different materials, which NANONET is all familiar with, is down to the diversity of the intermediate filaments themselves and their associated proteins. Hair, hoof and skin are the product of either dead or dying cells, but inside living cells other intermediate filaments perform biomechanical, organisational, positioning, scaffolding, signalling and transport functions. The spectrum of potential applications for this group of proteins and their bioengineered derivatives is limited therefore only by our knowledge of the systems themselves and our own imagination and ingenuity to exploit their commercial potential. Intermediate filaments are 10 nm in diameter, but form filaments that are thousands times longer. The individual filaments themselves are extremely robust and have very attractive biomechanical properties such as the ability to withstand very large forces via their strain-hardening feature. Their large aspect ratio means that the individual filaments can traverse relatively large distances and therefore interconnect distant nodes - a feature that is key to any hardwiring applications. Derivatising intermediate filaments for nanowires or using our knowledge of their assembly for future biomimetics is a part of our *bioinspired* translation. The assembly of these filaments is extremely robust, requiring no special cofactors, enzymes or energy input. Assembly is driven by protein-protein interactions allowing the monomers to be engineered and organised into higher order filament structures by chemical facilitators. The filament monomers can be produced recombinantly in large quantities and certainly on an industrial scale, so that any emergent

technology is in principle not limited in availability or restricted by cost issues. The monomer is also a module that can be derivatised to alter the filament surface and therefore modify, extend and reprogramme the functionality of the intermediate filament. The monomers can be stored in a primed state so that assembly can be initiated either by changing the pH or salt concentration of the storage solution. The assembled filaments can be organised by fluid flow for bulk preparations or else individually on a nanoscale by optical tweezers and AFM technologies. There are therefore many potential applications for intermediate filaments as nanowires within the telecommunication industry and as biomimetics, catalysts and filtration aids in the bioprocessing sector as well as their potential medical applications in drug delivery, regenerative medicine and harnessing stem cell technologies.

Objective: To use the Action's new knowledge of intermediate filaments, their assembly and biomechanical properties to develop new technologies and materials and new industry-informed bioinspired materials for potential commercialisation.

Interactions with other Working Groups: Knowledge generated in WG2 will inform the potential nano-engineered products and materials derived from intermediate filaments to initiate discussion groups, information gathering and topic-directed conferences in WG5.

Working Groups 4: Intermediate filaments in health and disease

Rationale: Intermediate filaments are crucial for the mechanical integrity and biological function of many different tissues in the body. The different researchers working on intermediate filament in health, in disease models and in various clinical contexts will join efforts in this Working Group. Over the last decade intermediate filaments have also emerged as important signaling scaffolds and organizers, involved in regulating growth, defense, and survival signaling or triggering death machineries. This Working Group is aiming at examining the interrelationships between intermediate filaments and critical signaling pathways, with special emphasis on their roles in regulating survival, stress, death signaling. Elucidating these completely novel signaling functions is important for understanding the roles of intermediate filaments in normal development and homeostasis and in the numerous disease conditions involving intermediate filaments. The focus will be on the functions of vimentin, GFAP and nestin, which have key roles in the decision making of many different types of stem cells, but especially those of the nervous system, the myocardium, and the striated muscle.

Striated muscle is a highly organized tissue, with direct links between morphology and function and effective mechanochemical signalling between the contractile apparatus and the nucleus and other organelles. One of the goals of the Action is to understand the function of this continuous cytoskeleton, formed by desmin and other intermediate filaments, and their linker proteins such as plakins, in force transmission, mechanochemical signalling, and integration of organelle structure and function and how its disturbance leads to myocardial degeneration and heart failure.
Astrocytes execute many control functions in the central nervous system. These cells are also intimately involved in the pathogenesis of neurodegenerative diseases, such as Alzheimer's disease and amyotrophic lateral sclerosis and in the response to neurotrauma or stroke. Response in the intermediate filament cytoskeleton of the astrocyte is important in these processes. Glial fibrillary acidic protein (GFAP), vimentin, nestin and synemin are the main intermediate filament protein in astrocytes, and these proteins are highly regulated during development, aging and in neurodegenerative diseases. Reactive astrocytes and their intermediate filaments play a key role in the healing process after brain or spinal cord trauma, and in post-traumatic neuroprotection. Integration of neural transplants and neural stem cells can be largely improved by modulation of intermediate filaments in astrocytes. Reactive astrocytes and upregulation of intermediate filaments in astrocytes play also major role in ischemic stroke, and astrocyte intermediate filaments were identified as a potential target for pharmacological intervention in stroke.
Interestingly, the stem cells in the adult brain are recognized by a specialized intermediate filament cytoskeleton. Also distinct populations of potential neurogenic astrocytes can be identified by the expression of different GFAP-isoforms. One of the ideas is that the neurogenic potential of astrocytes is determined by the specialized GFAP-intermediate filament-cytoskeleton. The induction of different GFAP-isoforms in neurogenic astrocytes will soften the intermediate filament-cytoskeleton, which is critical to withstand the increased physical stress during cell migration. In addition, the distinct GFAP-intermediate filament-cytoskeleton will induce specific molecular pathways that will support the function of neurogenic astrocytes.
In conclusion, specialized cells express tissue-specific intermediate filaments and consequently mutations in intermediate filament genes cause a broad range of diseases, including neurological, heart and skin disorders. The resources and techniques available in NANONET concerning this WG are mouse and cell models for diseases, dedicated cell lines, molecular and cell biological

techniques, assays for mouse behaviour, functional assays for cells. Also the intermediate filament system seems to be critical in the cytoskeletal response to stress and thus might be central to the body's response to a disease.

Objective: To elucidate the function of intermediate filaments in health and disease as a prerequisite to target intermediate filaments for therapeutic intervention and to compensate for missing or abnormal intermediate filaments in patients with mutations.

Interactions with other Working Groups: Knowledge from WG2 is needed to provide a basic understanding of the mechanics of the intermediate filament network and knowledge generated in WG1 will help to understand the function of intermediate filaments in health and disease in WG 4. Information on the function of intermediate filaments in physiology and pathology gathered in WG4 will inform potential medical innovations in drug delivery, regenerative medicine and harnessing stem cell technologies via WG5.

Working Group 5: Outreach

Rationale: A key activity in NANONET is to create a broad awareness of the importance of biological and mechanical functions of intermediate filaments in cells. Stimulating exchange of knowledge and resources between the different Working Groups is an important step in initiating awareness among basic and medical researchers. The tasks of this Working Group are to integrate intermediate filament knowledge databases and information about participating research groups in a NANONET website. Also the interactions with other national and international research programmes will be coordinated by this WG. The Action aims at an invaluable resource of information for PhD-students, post-docs and established researchers. One of the first tasks of this WG is to appoint the Action's website coordinator who will have the responsibility to build and maintain the intermediate filament website. This website will be instrumental in presenting our scientific and technological progress for scientists and for the general public. It will disseminate information to all our current commercial contacts and serve to signpost other small and large commercial interests to NANONET. Expressions of interest from both companies and individuals will be logged via the website and announcements of meetings, conferences as well as workgroup contact details will be available on this site. Activity summaries will be available on this site for reference and audit purposes.

Objective: To implement and coordinate the Outreach activities.
Interactions with other Working Groups: This Working Group gets information from WG1-4.

E. ORGANISATION

E.1 Coordination and organisation

The Action will follow the rules and procedures of COST to establish a general management structure. The Management Committee (MC) will coordinate the Action and will be formed by at least one representative of each participating country and a representative of each Working Group (WG). The Chair, Vice-Chair, Working Group coordinators and all other functions will be elected at the first MC meeting. The Chair and Vice-Chair will be responsible for the general organisation of the Action and will represent the Action towards the EU Commission and the public. The five WGs will be created according to COST rules and based on the initial groups that participated in the preparation of the proposal. NANONET will encourage both female and Early-Stage Researchers to coordinate WGs and to take part in the MC. The Action aims at a balance between established and Early-Stage Researchers in the MC, as this will contribute to the build up of an international network for the younger scientists. If required the MC can instate new WGs and WG coordinators. The first MC meeting will serve as a kick-off meeting, to discuss and finalize the general objectives, goals and deliverables of the Action, and to inaugurate the WGs. The composition of the WGs will be discussed and approved at the first MC meeting. Subsequently, the MC will meet at least once a year to discuss and determine the various activities of the Action. The MC will decide about the distribution of funds according to the scientific and networking needs of the Action. Also, the MC will prepare the annual reports and the final report.
Training Schools will be established for young researchers (PhD student level and early postdoctoral level). They are envisaged for a period of 3-5 days, with experts from the NANONET network, their associated international research groups, and (non-)European experts in the field of intermediate filaments. An exchange programme will be developed as Short-Term Scientific Missions (STSMs**)** to allow young and established researchers to visit other groups from the participating laboratories and to learn a specific method or get acquainted to another research area which is relevant for their studies. For optimally economizing costs and time, the annual meetings, joint international meetings and MC meetings will be combined and held at the same location and on adjacent or the same days.

E.2 Working Groups

Initially NANONET will implement the following Working Groups (See D):

- **WG1 – Mechanotransduction and cell signalling**
- **WG2 – Nanomechanical properties and impact on cell mechanics**
- **WG3 – Translation to bio-inspired materials**
- **WG4 – Intermediate filaments in health and disease**
- **WG5 – Outreach**

These Working Groups are open to all scientists interested in the subject and participating in NANONET. Additional Working Groups can be formed during the course of this Action.
A Chair and Vice-chair will be elected by the MC at the kick-off meeting of NANONET. All members of each WG, except WG5, will meet once a year at the WG workshop. Interaction between researchers of WGs will occur through regular communications and researchers are encouraged to become members of more than one NANONET WG.
WG5 will start with implementing the NANONET website at the NANONET kick-off meeting. WG1-4 will provide WG5 with regular updates of research progress and outreach activities. All WGs will connect to WG5, which will coordinate and facilitate outreach and dissemination activities.

E.3 Liaison and interaction with other research programmes

Liaisons with other programs, organisation and industry will be managed by WG5.
Many of the initial partners are involved in FP6 and FP7 programs, however these programs do not comprise a funded EU program that is focused on intermediate filaments. NANONET aims at initiating intermediate filament centered international collaborative research programs, including FP7 programs.
Interactions with national and international research programs will be implemented though the outreach activities that are going to be carried out in NANONET, such as scientific meetings, training workshops and the participation of international experts (also from non-COST countries) in meetings and workshops.

E.4 Gender balance and involvement of Early-Stage Researchers

This COST Action will respect an appropriate gender balance in all its activities. The Action will also be committed to involve Early-Stage Researchers. NANONET will actively pursue a balance in gender and in the participation of established and ESRs in the MC and WGs.

Female Scientists**:** In order to achieve an equitable gender balance in the proposed Action, NANONET will specifically encourage highly qualified women to participate. The Action will actively support the career perspectives of the women in the participating teams, by taking into account the gender balance during meetings and making sure that female scientist are invited to give lectures at workshops and meetings organized by NANONET.

Early-Stage Researchers**:** A special exchange and training school programme will be established for young researchers. It will target PhD students in their final years and ESRs at the postdoctoral level. Six of the 27 experts from 11 COST countries, who have been consulted during writing of this proposal, are female. Eight of the 27 experts are early stage researchers. At the moment of submission 8 males and 6 females are willing to participate in the MC, 10 are established and 4 are ESRs.

F. TIMETABLE

The total duration of the COST Action will be four years. The timeline of the NANONET activities is shown in the following table.

Activity	Year 1	Year 2	Year 3	Year 4
Kick-off meeting	Q1			
Inauguration of MC	Q1			
Inauguration of WGs	Q1			
WG meetings (definition of tasks, deliverables and Milestones, determining dates and topics of workshop)	Q2-3	Q3	Q3	Q3
MC meeting (reports, final definition of tasks)	Q3	Q3	Q3	Q3
Work shops	Q1-4	Q1-4	Q1-4	Q1-4
MC meeting (reports, final definition of tasks)	Q3	Q3	Q3	Q3
Training school		Q3		Q3
Annual NANONET strategic meeting	Q3	Q3	Q3	Q3
STMS	Q1-4	Q1-4	Q1-4	Q1-4
NANONET website – setting up	Q1-4			
NANONET website – updating and extending		Q1-4	Q1-4	Q1-4
Final report and publication				Q4
Final conference				Q3

G. ECONOMIC DIMENSION

The following COST countries have actively participated in the preparation of the Action or otherwise indicated their interest: Austria, Czech Republic, Finland , Germany, Greece, Israel, Netherland, Slovenia, Sweden, Switzerland, United Kingdom. On the basis of national estimates, the economic dimension of the activities to be carried out under the Action has been estimated at 44

Million € for the total duration of the Action. This estimate is valid under the assumption that all the countries mentioned above but no other countries will participate in the Action. Any departure from this will change the total cost accordingly.

H. DISSEMINATION PLAN

H.1 Who?

The participating scientists will publish their results on intermediate filament cytoskeleton related research in peer reviewed journals and present their results at international meetings and with interested commercial partners. These scientists have a well proven track record in high impact scientific research. Furthermore, the MC and the Working Groups will work out a dissemination plan to publish the specific results of their meetings, making available summaries on the NANONET website. This plan will be part of the deliverables and milestones.

H.2 What?

The main results of NANONET will be new findings by the participating scientific groups, which will be published in peer reviewed journals, the organisation of international meetings, and outreach via the NANONET website.

H.3 How?

The scientific results of the NANONET network will be published in scientific journals, presented at scientific meetings and filed as patents to protect commercially exploitable findings. Commercial Disclosure Agreements will be put in place to protect academic-industrial partnerships. A final report will be created for the EC containing suggestions for future funding programmes to support the intermediate filament research in Europe. In addition, the NANONET website will be the platform for information about the NANONET network.